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ALKALOIDAL AND METALLIC PRECIPITATION OF CEREBROSPINAL FLUID IN THE DIAGNOSIS OF MENINGITIS

STUDIES IN MENINGITIS. II

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The chemical tests that may be of diagnostic value in the differentiation of one form of meningitis from another are not numerous. The diagnosis as to the type of meningitis is based on bacteriologic findings generally. The bacteriologic tests when positive establish a definite diagnosis, but when negative they neither confirm nor rule out any disease, and it is left to make the diagnosis on the clinical and chemical data.

There are certain well-known diagnostic points that are of value in differentiating meningitis from nonmeningitic conditions, and one type of the disease from another.

In tuberculous meningitis, the spinal fluid is increased both in amount and pressure. The globulin reactions (Noguchi, Ross Jones, Nonne) are positive. The permanganate index is raised and the cells, which consist principally of lymphocytes, are increased in number. Most conclusive in this form of meningitis, is, of course, the demonstration of tubercle bacilli in the spinal fluid.

In epidemic meningitis, the fluid is almost always turbid and increased in amount and pressure, all globulin tests are positive, and the cells, which are mainly polymorphonuclear, are increased in number. The conclusive test in this form is the demonstration of meningococci in the fluid by means of smears and cultures. The same diagnostic means are employed in suppurative nonepidemic meningitis. Yet, in spite of all this, the diagnosis of the form of meningitis may remain unsettled. It is well-known that not only is it hard to find tubercle bacilli in smears of the fluid, but that often the globulin tests are negative until very late in the course of the disease. As to epidemic meningitis, sometimes the bacteria are few in number and sometimes they are not found at all. To grow the meningococci in cultures may be difficult, at times impossible. We therefore wish to present some results of chemical studies that may prove of corroborative value in differentiating meningitis from other conditions, and in separating the different forms from one another, principally tuberculous from epidemic meningitis.

Of the chemical and physico-chemical characteristics of spinal fluid, two stand out prominently in pathologic conditions, namely, protein content, and variation in the reaction of the fluid, or its hydrogen-ion concentration. As stated, globulin is increased in all meningeal inflammations. However, the amount of protein contained in various conditions gives us no criteria as to

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the specific nature of the disease. Mestrezat[1] showed that in epidemic meningitis albumin may vary all the way from 0.15% to 0.85%, and in tuberculous meningitis from 0.12% to 0.56%. Thus it is evident that although epidemic meningitis usually has a higher protein content than tuberculous meningitis, the latter may at times have a higher protein content than the former. It is plain then that the protein content alone is not sufficient evidence on which to build the differentiation of the disease.

The other factor is the variation of the reaction of the fluid in different conditions, some fluids giving a high acidity and others a low one. Levinson[2] found that spinal fluid is alkaline to methyl red, that in tuberculous meningitis it gives practically the same index of alkalinity as normally, and that in epidemic and pneumococcus meningitis the fluid is much less alkaline than normally. Levinson's work, however, was done with fluid that had been standing for some time before being examined. Kopetzky[3] showed that normal fluid is neutral to litmus, but that in tuberculous and epidemic meningitis the fluid is acid to phenolphthalein, slightly less so in the tuberculous than in the epidemic disease.

TABLE 1

SHOWING CATAPHORESIS OF PROTEINS; FIGURES TAKEN FROM MICHAELIS[5]

Substances	H^+	Moves to
Casein	4.9×10^{-5}	Cathode
	4.1×10^{-5}	Stand still
	1.3×10^{-5}	Anode
Serum Albumin	$1.2 \times 10^{-4} — 2.1 \times 10^{-5}$	Cathode
	$2.0 \times 10^{-5} — 1.9 \times 10^{-5}$	Stand still
	$1.1 \times 10^{-6} — 1.9 \times 10^{-5}$	Anode
Oxyhemoglobin	2.4×10^{-7}	Cathode
	1.2×10^{-7}	Anode
Gelatin	1.5×10^{-5}	Anode
	$1.2 \times 10^{-5} — 3.5 \times 10^{-6}$	Stand still
	3.9×10^{-5}	Cathode

As stated by Levinson (Page 556), we found that on immediate examination the H-ion concentration of fluid from tuberculous meningitis is exactly like that in nonmeningitic conditions and that H-ion concentration in epidemic meningitis is usually slightly higher than normal, remaining high for some time with a tendency to increase as the fluid stands. We also tried to show that the mechanism responsible for the high acidity in the fluid in epidemic meningitis after standing is the formation of some acid in the fluid, possibly lactic acid. One thing, however, seems certain, namely, the difference in the acidity of the fluid in tuberculous and epidemic meningitis. Sooner or later, a difference in the acidity of the two forms is apt to assert itself. It is this variation in acidity that we shall discuss now.

If the difference in the reaction of the different fluids is great enough, we should be able to distinguish their protein in a variety of ways, even if their protein concentration might be the same. To make our point clearer, let us discuss briefly the precipitation reactions of the protein: For a more detailed discussion, see Mathews.[4]

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[1] Le Liquide Cephalo-Rachidien, 1912.

[2] Arch. Pediat., 1916, 33, p. 241.

[3] Manhattan Eye, Ear and Throat Hospital Reports, 1913, 14.

It is well known that the electrical charge of a protein depends on the reaction of the medium. In acid solution, proteins become electropositive, and in alkaline solutions they become electronegative, as shown in Table 1. Thus, one of the ways of precipitating protein is to let it combine with some radicle to form an insoluble protein salt. From the standpoint of the electrical charge of the protein, such a protein salt is necessarily either a positive protein radicle forming a protein salt with negatively charged ions (alkaloidal precipitants), such as tungstic, picric, tannic acid and the like, or a negatively charged protein combining with a positively charged metal (metallic precipitants) such as Cu, Ag, Hg, Zn and Pb. If these protein salts are sufficiently insoluble, a precipitate will come down.

We thought it would be of interest to ascertain whether the difference in acidity of the spinal fluid under various conditions is great enough to produce different electrical states of the protein in the fluids. If it should be, it would be worth while to determine whether, by means of proper precipitants, we can distinguish one type of disease from the other.

EXPERIMENTS WITH CATAPHORESIS

Our first experiments were made to determine whether we could demonstrate electrically the existence of differently charged proteins in the spinal fluid of the two diseases. The apparatus we used was the one recommended by Michaelis[5] (Fig. 1). The fluids were placed in 3, care being taken to see that there was no bubble inside the core of the stopcocks. Tubes 2 and 4 were filled with 3% sulphosalicylic acid, and the upper portions (1 and 5), were filled with distilled water. To one electrode we added $CuCl_2$ and to the other Nacl; we put a silver electrode in the anode and a copper electrode in the cathode. The poles were connected to a light circuit with 110 volt constant current, with an ordinary lamp in the circuit as resistance. The object was to determine the pole to which the protein would move. The migration of the protein from the spinal fluid was detected by the formation of a precipitate with sulphosalycilic acid in the arm. This method of course is necessarily crude. For an accurate test it would be necessary that the H-ion concentration, the osmotic pressure of the precipitants and of spinal fluid are the same for each experiment—a procedure almost impossible on account of the variation in the fluid. Furthermore, under these conditions, positively charged protein has a better chance to be precipitated at the cathode, while negatively charged protein, which moves to the anode, must change its charge at the anode in order to combine with sulphosalicylic acid. In other words, the amount of negatively charged protein to be precipitated at the anode depends not only on the amount of protein that moves to the anode, but also on the strength of the acidity of the precipitating agent at the anode.

However crude this method may be, it is convenient for detecting the charge of protein in spinal fluid. On this account, we used cataphoresis for testing the fluid of epidemic meningitis which shows a high acidity (Table 2).

As seen from the table, it is evident that in epidemic meningitis a considerable amount of protein moves toward the cathode, showing the presence of positively charged protein. In tuberculous meningitis we determined the charge of the protein of the fluid and found that in most cases it was at the anode, proving that there is more negatively charged protein in the fluid in this disease (Table 3). In nonmeningitic fluids there is very little precipitation in cataphoresis, only a slight diffusion being seen at one side or the other. Thus, it is possible to demonstrate that the existing variation in H-ion concentration of the spinal fluid is great enough to produce the different states of electrical charges of the protein. After this is established, it should be further possible to choose proper precipitants to distinguish the various forms of meningitis. In other words, if it is true that the variations are due to the differently charged proteins, we ought to be able to find more precipitate with a metallic precipitant than with an alkaloidal precipitant in tuberculous fluid, and in epidemic fluid the reverse would be the case.

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[4] Physiological Chemistry, 1916.

[5] Die Wasserstoffionenkonzentration, 1914.

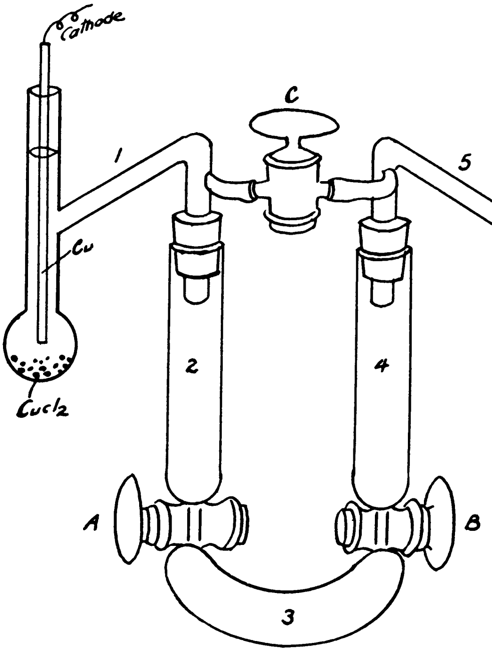

Fig. 1.—Apparatus for cataphoresis of proteids, after Michaelis, actual size.

The method we adopted was the following: We selected a 1% solution $HgCl_2$ as the metallic precipitant, and a 3% sulphosalicylic acid as the alkaloidal precipitant.

We put 1 c.c. of a 3% sulphosalicylic acid in a small test tube about 7-8 mm. wide and 1 c.c. of a 1% mercuric chlorid in another tube of the same dimension. We added 1 c.c. of the same spinal fluid to each tube, believing that the more acid the fluid, the more precipitation there would be in the tube containing the sulphosalicylic acid, and the more alkaline the fluid the more precipitate there would be in the tube containing the mercuric chlorid.

We found that normal fluid gives only slight turbidity with sulphosalicylic acid and is clear with mercuric chlorid. The fluid on standing shows a little sediment at the bottom of the tube, the precipitate in the $HgCl_2$ usually measuring 2.5-4 mm. in height and that in the sulphosalicylic acid 1-3 mm. (Table 4).

TABLE 2

RESULTS OF CATAPHORESIS ON FLUIDS FROM EPIDEMIC MENINGITIS

Number	Interval	Cataphoresis		Remarks
		Cathode	Anode	
1	1 day	—»	↓	
1	1 day	—»	↓	
2	20 hours	—»	↓	
2	3 days	—»	↓	
3	3 days	—»	↓	
3	2 days	↓	—»	Fluid bloody
3	3 hours	—»	↓	
4	8 hours	↓	—»	Unusually alkaline fluid; cases brought in from another hospital; administration of serum not ascertained
5	?	↓	—»	
6	....	—»	↓	
6	17 hours	—»	↓	
7	....	—»	↓	
7	2 days	—»	↓	

—» indicates heavy precipitate
↓ slight precipitate.

TABLE 3

RESULTS OF CATAPHORESIS ON FLUID FROM CASES OF TUBERCULOUS MENINGITIS

Number	Time after Withdrawal	Cataphoresis	
		Cathode	Anode
1	2 hours	↓	—»
1	2 days	—»	—»
1	2 days	↓	—»
2	2 hours	↓	—»
2	3 hours	↓	—»
3	1 day	↓	—»
4	3 hours	↓	—»
5	24 hours	↓	—»

—» indicates heavy precipitate
↓ slight precipitate.

In tuberculous meningitis the sulphosalicylic acid gives a turbid precipitate immediately which settles to the bottom in several hours to a height of 3-6 cm. The mercuric chlorid is clear at first, but in 12-24 hours it settles in a gelatinous precipitate measuring 6-20 mm. (Table 5).

TABLE 4

COMPARISON OF AMOUNT OF SEDIMENT FROM THE METALLIC AND ALKALOIDAL PRECIPITATION WITH NONMENINGITIC FLUIDS

Number	Diagnosis	Measurements of the Depth of Sediment in MM. after 24 Hours	
		$HgCl_2$	Sulphosalicylic Acid
1	Psychosis	3	2
2	Tic.	4	3
3	General paresis	4½	2
4	Delirium tremens	3	1
5	General paresis	3½	2
6	Psychosis	3	2
7	General paresis	3½	2
8	General paresis	3	0
9	Psychosis	4	3
10	Psychosis	4	0
11	Psychosis	4	2
12	Meningism	3	2
13	Dementia praecox	3	0
14	Alcoholic	3	2
15	Psychosis	3	2
16	....	4	2
17	Psychosis	3	1
18	Psychosis	2½	2
19	Meningism	3	2

TABLE 5

AMOUNT OF SEDIMENT FROM THE METALLIC AND ALKALOIDAL PRECIPITATION FROM FLUID OF TUBERCULOUS MENINGITIS

Number	Measurements of the Depth of Sediment in MM. after 24 Hours	
	$HgCl_2$	Sulphosalicylic Acid
1	10	4
1	15	4
1	10	3½
2	11	5
2	6	3
3	7	3
4	9	6
4	13	5
4	11	7
5	10	4
6	6	3
6	10	4
7	20	5

In epidemic meningitis before serum is given the sulphosalicylic acid produces a very marked turbidity, immediately forming a heavy precipitate. In several hours the precipitate settles to a height of from 7 to 20 mm. The mercuric chlorid, on the other hand, is clear immediately and shows a very slight sediment on standing, ranging from 1 to 7 mm. in height, which in comparison with that produced by sulphosalicylic acid, is at a ratio of from 1:3, or even 1:5 (Table 6).

TABLE 6

MEASUREMENT OF SEDIMENT FROM METALLIC AND ALKALOIDAL PRECIPITATION FROM FLUID IN EPIDEMIC MENINGITIS BEFORE SERUM WAS GIVEN

Number	Measurements of the Depth of Sediment in MM. after 24 Hours	
	$HgCl_2$	Sulphosalicylic Acid
1	1	8
2	2	7
3	1	20
4	1	10
5	7	20

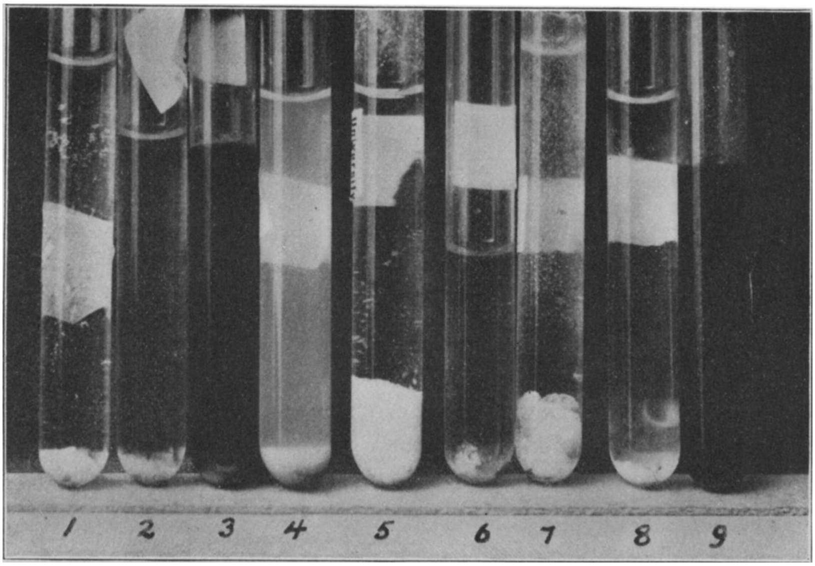

Fig. 2.—Photograph showing the typical ratio of precipitates by the 2 precipitants.

1.—1 c.c. of nonmeningitic fluid + 1 c.c. of 1 % $HgCl_2$.
2.—1 c.c. of nonmeningitic fluid + 1 c.c. of 3 % sulphosalicylic acid.
3.—1 c.c. of nonmeningitic fluid + phenolphthalein.
4.—1 c.c. of fluid from epidemic meningitis + 1 c.c. of $HgCl_2$.
5.—1 c.c. of fluid from epidemic meningitis + 1 c.c. of sulphosalicylic acid.
6.—1 c.c. of fluid from epidemic meningitis + phenolphthalein.
7.—1 c.c. of fluid from tuberculous meningitis + 1 c.c. of $HgCl_2$.
8.—1 c.c. + 1 c.c. of sulphosalicylic acid.
9.—1 c.c. + phenolphthalein.

It should be noted that there is a slight excess of precipitate in the $HgCl_2$ tube over the sulphosalicylic tube in nonmeningeal fluids; that there is a much greater excess of $HgCl_2$ precipitate in tuberculous meningitis; and that in epidemic fluid the sulphosalicylic precipitate is in excess over the $HgCl_2$.

After serum is given the difference between the two precipitants is not so marked as before but it is still marked enough to distinguish the fluid from the fluid of tuberculous meningitis, if the patient still shows marked meningeal symptoms.

It should be emphasized here that the principal feature in the precipitation experiment is not the amount of sediment in the tube, for this depends on the protein contents which vary considerably in different cases, but the important point is the ratio between precipitates obtained by mercuric chlorid and sulphosalicylic acid, a matter that depends chiefly on the H-ion concentration of the fluid.

It is interesting that the precipitation results are fairly constant regardless of the time that the fluid has been standing after withdrawal from the body. Since the H-ion concentration varies greatly on standing, but not very much on immediate examination, it may seem rather contradictory at first sight to have the precipitation not vary in different conditions. However, as we have tried to show, the gradual increase in the alkalinity of the fluid of tuberculous meningitis and of normal fluid is due to the loss of CO_2, while in epidemic meningitis there is an acid other than CO_2 that is responsible for the gradual increase. It seems probable that when sulphosalicylic acid is added to fresh fluid in tuberculous meningitis, it hastens the escape of CO_2, a condition similar to the gradual loss of CO_2 on standing; while in epidemic meningitis the loss of CO_2 is not greatly affected by sulphosalicylic acid as the main acidity is probably due to a nonvolatile acid. For this reason the mercuric chlorid precipitate does not settle immediately as the H-ion concentration is high at the beginning. Thus, in tuberculous meningitis where the precipitate occurs it takes some time before it comes down.

Three typical results, representing nonmeningitic, tuberculous and epidemic meningitis, showing ratios of the precipitates obtained by two types of precipitating agents are shown in Figure 2.

SUMMARY

By a study of cataphoresis it is possible to show that there is a difference in the electrical charge of the protein in the fluids of the different forms of meningitis, the fluid in cases of epidemic meningitis containing more positively charged protein and the fluid in cases of tuberculous meningitis containing more negatively charged protein.

A convenient way of making a differential diagnosis of meningitis is to precipitate the spinal fluid by an alkaloidal, 3% sulphosalicylic acid, and a metallic, 1% mercuric chlorid, precipitant. All meningitic fluids show turbidity with the alkaloidal precipitant as soon as the test is made, thus indicating an inflammation of the meninges. On letting the precipitated fluid stand, a further differentiation is made by observing the ratio between the alkaloidal and metallic sediments. In normal fluid the sediment produced by either precipitant is slight, the sulphosalicylic acid sediment usually measuring 1-3 mm. in height and the mercuric chlorid 2-4 mm. in height. In tuberculous meningitis the sediment of the mercuric chlorid precipitation is usually twice the height of the sulphosalicylic acid precipitation. In epidemic meningitis, on the other hand, the mercuric chlorid precipitation is slight and the sulphosalicylic acid precipitation is marked, the ratio of sulphosalicylic acid to mercuric chlorid ranging from 2: 1 to 3: 1. The ratio in epidemic meningitis is disturbed after serum is given.